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Establishment of gene transfer and gene silencing methods in a desiccation-tolerant cell line, Pv11

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Abstract

© 2016, Springer Japan.Larvae of the African midge Polypedilum vanderplanki show extreme desiccation tolerance, known as anhydrobiosis. Recently, the cultured cell line Pv11 was derived from this species; Pv11 cells can be preserved in the dry state for over 6 months and retain their proliferation potential. Here, we attempted to expand the use of Pv11 cells as a model to investigate the mechanisms underlying anhydrobiosis in P. vanderplanki. A newly developed vector comprising a constitutive promoter for the PvGapdh gene allowed the expression of exogenous proteins in Pv11 cells. Using this vector, a stable Pv11 cell line expressing green fluorescence protein (GFP) was established and retained desiccation tolerance. Gene silencing with GFP-specific siRNAs significantly suppressed GFP expression to approximately 7.5–34.6% of that in the non-siRNA-transfected GFP stable line. Establishment of these functional assays will enable Pv11 cells to be utilized as an effective tool to investigate the molecular mechanisms underlying anhydrobiosis.

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Keywords

Anhydrobiosis, Desiccation tolerant cells, Gene expression system, RNAi

References

[1] Cornette R, Kanamori Y, Watanabe M et al (2010) Identification of anhydrobiosis-related genes from an expressed sequence tag database in the cryptobiotic midge Polypedilum vanderplanki (Diptera; Chironomidae). J Biol Chem 285:35889–35899. doi:10.1074/jbc.M110.150623

[2] Ducrest AL, Amacker M, Lingner J, Nabholz M (2002) Detection of promoter activity by flow cytometric analysis of GFP reporter expression. Nucleic Acids Res 30:e65. doi:10.1093/nar/gnf064

[3] Erkut C, Penkov S, Khesbak H et al (2011) Trehalose renders the dauer larva of Caenorhabditis elegans resistant to extreme desiccation. Curr Biol 21:1331–1336. doi:10.1016/j.cub.2011.06.064

[4] Gusev O, Suetsugu Y, Cornette R et al (2014) Comparative genome sequencing reveals genomic signature of extreme desiccation tolerance in the anhydrobiotic midge. Nat Commun 5:4784. doi:10.1038/ncomms5784

[5] Hatanaka R, Hagiwara-Komoda Y, Furuki T et al (2013) An abundant LEA protein in the anhydrobiotic midge, PvLEA4, acts as a molecular shield by limiting growth of aggregating protein particles. Insect Biochem Mol Biol 43:1055–1067. doi:10.1016/j.ibmb.2013.08.004

[6] Kikawada T, Nakanahara Y, Kanamori Y et al (2006) Dehydration-induced expression of LEA proteins in an anhydrobiotic chironomid. Biochem Biophys Res Commun 348:56–61. doi:10.1016/j.bbrc.2006.07.003

[7] Li S, Chakraborty N, Borcar A, Menze MA, Toner M, Hand SC (2012) Late embryogenesis abundant proteins protect human hepatoma cells during acute desiccation. Proc Natl Acad Sci USA 109:20859–20864. doi:10.1073/pnas.1214893109

[8] Naito Y, Yoshimura J, Morishita S, Ui-Tei K (2009) siDirect 2.0: updated software for designing functional siRNA with reduced seed-dependent off-target effect. BMC Bioinform 10:392. doi:10.1186/1471-2105-10-392

[9] Nakahara Y, Imanishi S, Mitsumasu K et al (2010) Cells from an anhydrobiotic chironomid survive almost complete desiccation. Cryobiology 60:138–146. doi:10.1016/j.cryobiol.2009.10.004

[10] Okada J, Kikuta S, Gusev O et al (2015) Construction of optimized CRISPR/Cas system to reveal the mechanisms of anhydrobiosis in the Sleeping Chironomid. Cryobio Cryotech 61:69–73

[11] Sakurai M, Furuki T, Akao K et al (2008) Vitrification is essential for anhydrobiosis in an African chironomid, Polypedilum vanderplanki. PNAS 105:5093–5098. doi:10.1073/pnas.0706197105

[12] Shimizu T, Kanamori Y, Furuki T et al (2010) Dessication-induced structuralization and glass formation of group 3 Late Embryogenesis Abundant protein model peptides. Biochemistry 49:1093–1104. doi:10.1021/bi901745f

[13] Ui-Tei K, Naito Y, Takahashi F et al (2004) Guidelines for the selection of highly effective siRNA sequences for mammalian and chick RNA interference. Nucleic Acids Res 32:936–948. doi:10.1093/nar/gkh247

[14] Watanabe M (2006) Anhydrobiosis in invertebrates. Appl Entomol Zool 41:15–31

[15] Watanabe M, Kikawada T, Okuda T (2003) Increase of internal ion concentration triggers trehalose synthesis associated with cryptobiosis in larvae of Polypedilum vanderplanki. J Exp Biol 206:2281–2286

[16] Watanabe K, Imanishi S, Akiduki G, Cornette R, Okuda T (2016) Air-dried cells from the anhydrobiotic insect, Polypedilum vanderplanki, can survive long term preservation at room temperature and retain proliferation potential after rehydration. Cryobiology 73:93–98. doi:10.1016/j.cryobiol.2016.05.006